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Evaluation of Amphotericin B Interpretive Breakpoints for *Candida* Bloodstream Isolates by Correlation with Therapeutic Outcome

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One hundred seven *Candida* bloodstream isolates (51 *C. albicans*, 24 *C. glabrata*, 13 *C. parapsilosis*, 13 *C. tropicalis*, 2 *C. dubliniensis*, 2 *C. krusei*, and 2 *C. lusitaniae* strains) from patients treated with amphotericin B alone underwent in vitro susceptibility testing against amphotericin B using five different methods. Fifty-four isolates were from patients who failed treatment, defined as death 7 to 14 days after the incident candidemia episode, having persistent fever of ≥5 days' duration after the date of the incident candidemia, or the recurrence of fever after two consecutive afebrile days while on antifungal treatment. MICs were determined by using the Clinical Laboratory Standards Institute (formally National Committee for Clinical Laboratory Standards) broth microdilution procedure with two media and by using Etest. Minimum fungicidal concentrations (MFCs) were also measured in two media. Broth microdilution tests with RPMI 1640 medium generated a restricted range of MICs (0.125 to 1 μg/ml); the corresponding MFC values ranged from 0.5 to 4 μg/ml. Broth microdilution tests with antibiotic medium 3 produced a broader distribution of MIC and MFC results (0.015 to 0.25 μg/ml and 0.06 to 2 μg/ml, respectively). Etest produced the widest distribution of MICs (0.094 to 2 μg/ml). However, none of the test formats studied generated results that significantly correlated with therapeutic success or failure.

The Clinical Laboratory Standards Institute (CLSI; formally National Committee for Clinical Laboratory Standards) has developed a standardized broth dilution procedure for in vitro susceptibility testing of *Candida* species against amphotericin B, flucytosine, fluconazole, and itraconazole (8). Although this method has proven to be reliable and reproducible, it generates a restricted range of amphotericin B MICs, precluding reliable discrimination between susceptible and resistant isolates of *Candida* species and preventing the development of interpretive MIC breakpoints for in vitro testing (1, 9, 15). Testing using antibiotic medium 3 (AM3), instead of RPMI 1640 medium, has been reported to generate a broader range of MIC results, improving the detection of isolates less susceptible to amphotericin B (13). However, AM3 is not a defined medium, and lot-to-lot variation may reduce the reproducibility of the MIC results (7). Other reports have indicated that the Etest provides better discrimination between amphotericin B-susceptible and -resistant *Candida* isolates than does the CLSI reference method and have suggested that MICs obtained by this test are more predictive of treatment outcome (2, 4, 12, 17). Previous studies have also suggested that the minimum fungicidal concentration (MFC) may be a more accurate measure of microbial susceptibility to fungicidal agents such as amphotericin B (9).

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In this investigation, we used five different methods of in vitro amphotericin B susceptibility testing, including MIC and MFC determination in two media and the Etest, in an attempt to determine whether results from these tests could be correlated with treatment outcomes in patients with *Candida* bloodstream infections that had been treated with amphotericin B alone. These patients were identified as part of a prospective population-based active surveillance program for candidemia conducted between 1998 and 2000 (6).

MATERIALS AND METHODS

Isolates and data collection. One hundred seven *Candida* bloodstream isolates were collected between 1 October 1998 and 30 September 2000 as a part of a population-based, active laboratory surveillance conducted in Connecticut and Baltimore City/County, Md. (6). All patients had been treated for at least 7 days with amphotericin B alone (conventional or lipid formulations). Patients who died before day 7 were excluded. A standardized data collection form was used to abstract demographics, clinical characteristics, underlying conditions, and outcome from medical records. Treatment failure was defined as death 7 to 14 days after the incident candidemia episode, having persistent fever of ≥5 days' duration after the date of the incident candidemia, or demonstrating the recurrence of fever after two consecutive afebrile days while on antifungal treatment that is unexplained by another cause (e.g., bacteremia); treatment success was defined as having none of the previous criteria.

Species identification was confirmed by standard methods, including characteristic growth on Chromagar *Candida* plates, API 20C biochemical profiles, and morphological appearance on Dalmau cornmeal agar plates. Isolates of *C. dubliniensis* were identified by PCR amplification of a region containing the novel *C. dubliniensis* group I intron (3). The 107 isolates comprised 51 *C. albicans*, 24 *C. glabrata*, 13 *C. parapsilosis*, 13 *C. tropicalis* isolates and two each of *C. dubliniensis*, *C. krusei*, and *C. lusitaniae*. One previously defined amphotericin B-susceptible (CL524) and two previously defined amphotericin B-resistant isolates

(MY1012 and CL2887), from which well-documented data from in vivo and in vitro studies are available (13), were received from John Rex and included as controls in each of the five in vitro methods. Prior to antifungal susceptibility testing, each isolate was subcultured at least twice on Sabouraud dextrose agar (SDA) plates (Difco Laboratories, Detroit, MI). The QC strain, *C. krusei* ATCC 6258 and the reference strain, *C. parapsilosis* ATCC 90018, were included in each batch of susceptibility tests to ensure quality control.

Antifungal agent. A concentrated stock solution of amphotericin B (analytical grade powder; Sigma Aldrich, St. Louis, MO) was dissolved in dimethyl sulfoxide; the solution was diluted with dimethyl sulfoxide and then with the appropriate test medium. Amphotericin B was tested over a concentration range of 0.015 to 8 μg/ml.

Broth microdilution susceptibility testing. MICs were determined according to the CLSI M27-A2 microdilution procedure (8) by using two testing media: (i) RPMI 1640 medium with L-glutamine and without bicarbonate (Sigma Aldrich), buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS; Sigma), and (ii) AM3 medium (Difco) supplemented with 2% dextrose. Cell suspensions of *Candida* species were adjusted to match the turbidity of a 0.5 McFarland standard when a spectrophotometer set at 530 nm was used. Cell suspensions were further diluted 1:1,000 in test medium, and 100 μl was added to each well of the microtiter plate to yield a final inoculum concentration of 0.5×10^3 to 2.5×10^3 cells/ml. The plates were incubated at 35°C and read visually after 48 h. The MIC was defined as the lowest drug concentration at which there was complete inhibition of growth.

Determinations of MFC. To accurately assess the MFC of an antifungal agent at a ≥99.9% kill, a starting inoculum size of at least 10^4 cells/ml is required. For MFC determination, broth microdilution tests were set up as described above except a higher inoculum concentration, 1×10^4 to 2×10^4 cells/ml, was used. After 48 h of incubation at 35°C, the entire content (200 μl) from each well with no visible growth was homogenized with a micropipette and subcultured onto two SDA plates (100 μl/plate). To prevent antifungal carryover effects, inocula were allowed to soak into the agar for 15 min before spreading the cells away from the spot. The SDA plates were incubated at 35°C for 48 h, and the MFC was defined as the lowest drug concentration that yielded ≤1 colony on both plates.

E-test MIC testing. The MICs of amphotericin B were determined by Etest according to the manufacturer's instructions (Etest technical guide number 4; antifungal susceptibility of yeasts; AB BIODISK, Solna, Sweden). Briefly, yeast suspensions were adjusted to match the turbidity of a 0.5 McFarland standard when a spectrophotometer set at 530 nm was used. The medium used was RPMI 1640 agar (1.5%) supplemented with dextrose (2%) and buffered to pH 7.0 with MOPS. The plates were inoculated by dipping a sterile cotton swab into the appropriate cell suspension and streaking it across the entire surface of the agar in three directions. The plates were dried for 15 min before the Etest strips were applied. The plates were incubated at 35°C and read visually after 48 h. The Etest MIC was defined as the drug concentration at which the border of the elliptical zone of complete inhibition intersected the scale on the antifungal test strip.

Identification of candidate interpretive breakpoints. Because accepted interpretive breakpoints have not been established for amphotericin B, we identified candidate breakpoints for inclusion into multivariable modeling of treatment failure by using three different approaches. First, if the geometric mean or median MIC or MFC values for isolates from treatment failures were significantly higher than the corresponding values for isolates from treatment successes, a candidate breakpoint was identified at the MIC or MFC value separating the two groups. Second, receiver operator characteristic (ROC) curves were prepared for each test method. At each potential breakpoint for each testing method, the sensitivity was plotted on the *y* axis and 1 minus the specificity was plotted on the *x* axis. Sensitivity and specificity were determined by using the following formulas: sensitivity = the number of resistant treatment failures/the total number of failures; specificity = the number of susceptible treatment successes/the total number of successes. If a test value plotted on the ROC curve demonstrated a clear point of maximum sensitivity and minimum (1-specificity), this was identified as a suitable candidate breakpoint. Third, if no clear candidate breakpoint could be identified based on the previous two methods, breakpoints based on published data from animal models of infection and studies of human patients with candidiasis were used.

Clinical correlations of in vitro susceptibilities. We modeled the predictors of treatment failure to correlate susceptibility test results with clinical outcome; exposures included in vitro resistance as defined by the candidate breakpoints, as well as clinical data available from surveillance. Variables significant at $P < 0.1$ on univariate analysis were included in multivariable models, with the criterion for staying in the model set at $P = 0.05$. Five different multivariable models were created, with a separate model for each susceptibility test method. We adjusted for potential confounding variables, such as age, the patient's underlying severity of illness, catheter removal, the presence of a subsequent bacteremia, and the type of *Candida* species. In addition, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each candidate breakpoint was determined. PPV and NPV were calculated as follows: PPV = the number of resistant treatment failures/the total number of resistant cases; NPV = the number of susceptible treatment successes/the total number of susceptible cases.

TABLE 1. Demographic and clinical characteristics of 107 patients treated with an amphotericin B formulation alone for ≥7 days

Characteristic	No. (%)
Median age	37 (range, 0–85)
Male gender	66 (62)
African-American race	53 (50)
Infant (<1 year)	33 (31)
Median APACHE II score	15 (range, 4–29)
In ICU at diagnosis[a]	56 (52)
Surgery in last 3 months	56 (52)
Neutropenia	15 (15)
Renal failure	29 (27)
HIV infection	9 (9)
Bone marrow or solid organ transplant recipient	7 (7)
Malignancy	20 (19)
Central venous catheter	93/98 (95)
Treatment	
Amphotericin B alone	83 (78)
Lipid formulation alone	11 (10)
Both amphotericin B and lipid	13 (12)
Death at days 7 to 14	14 (13)
Persistent fever for ≥5 days after candidemia	34/71 (48)
Recurrence of fever after ≥2 days on antifungal medication	29/70 (41)
Treatment failures	54 (51)

[a] ICU, intensive care unit.

Statistical analysis. Statistical analysis was performed by using SAS version 8.2 (SAS Institute, Cary, NC). The Fisher exact and Mantel-Haenzel chi-squared tests were used as appropriate for categorical variables. Continuous values with normal distribution were compared by using the Student *t* test, and median values were compared to the Wilcoxon two-sample test. A *P* value of <0.05 was considered statistically significant. Multivariable analyses were performed with the LOGISTIC procedure, using forward, backward, and stepwise elimination.

RESULTS

Patient characteristics. Table 1 summarizes the demographic and clinical characteristics of the 107 patients from our population-based surveillance program that were identified as having been treated with amphotericin B alone. A total of 83 case-patients had received conventional amphotericin B, 11 had received a lipid formulation, and 13 had received both conventional amphotericin B and a lipid formulation. A total of 13% of the case-patients died by day 14, 48% had a persistent fever for ≥5 days after candidemia, and 41% experienced recurrence of fever after having been on antifungal medications for at least 2 days. Overall, 54 (51%) treatment failures were observed.

The median age was 37 years (range, 0 months to 85 years), 33 case-patients were younger than 1 year old, and 66 were male. More than half of the case-patients were in an intensive care unit at diagnosis, and 52% had undergone surgery within the previous 3 months. As a part of treatment for the candidemia, all central venous catheters were removed or changed in 81 of the 93 (87%) case-patients with these catheters in place at the time of candidemia.

Distribution of amphotericin B MICs and MFCs. Broth microdilution testing with RPMI 1640 medium generated a narrow

TABLE 2. Distribution of amphotericin B MICs (A columns) and MFCs (B columns) as determined by broth microdilution testing with RPMI 1640 and AM3 media grouped by *Candida* species

Dilution (μg/ml)	No. of *Candida* isolates																					
	C. albicans (*n* = 51)				*C. glabrata* (*n* = 24)				*C. parapsilosis* (*n* = 13)				*C. tropicalis* (*n* = 13)				Others (*n* = 6)[a]				Total (*n* = 107)[b]			
	RPMI		AM3		RPMI		AM3		RPMI		AM3		RPMI		AM3		RPMI		AM3		RPMI		AM3	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
0.015	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	2, S	0
0.03	0	0	14	0	0	0	1	0	0	0	8	0	0	0	7	0	0	0	1	0	0	0	31	0
0.06	0	0	35	3	0	0	7	0	0	0	4	0	0	0	6	4	0	0	2	0	0	0	54	7, S
0.125	1	0	1	37	0	0	15	3	0	0	0	3	0	0	0	9	0	0	2	3	1	0	18	55
0.25	12	0	1	10	6	0	1	11	0	0	0	5	0	0	0	0	1	0	0	2	19	0	2	28
0.5	38	19	0	1	16	6	0	4	11	1	0	3	11	7	0	0	3	3	0	1	79	36	0	9
1	0	16	0	0	2	15	0	2	2	9	0	2	2	5	0	0	2	2	0	0	8, S	47, S	0, R2	4, R2
2	0	12	0	0	0	3	0	4	0	2	0	0	0	1	0	0	0	1	0	0	0, R1, R2	19, R2	0	4
4	0	4	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	5, R1	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
>8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	R1	R1

[a] *C. krusei* (*n* = 2), *C. dubliniensis* (*n* = 2), and *C. lusitaniae* (*n* = 2).
[b] R1, amphotericin B-resistant control isolate MY1012; R2, amphotericin B-resistant control isolate CL2887; S, amphotericin B-susceptible control isolate CL524.

range of four different MICs, ranging from 0.125 to 1 μg/ml; the corresponding MFC values ranged from 0.5 to 4 μg/ml (Table 2). Broth microdilution tests with AM3 produced a broader distribution of MIC and MFC results, ranging from 0.015 to 0.25 μg/ml and from 0.06 to 2 μg/ml, respectively (Table 2). Antifungal susceptibility testing by Etest produced the widest distribution of MICs, ranging from 0.094 to 2 μg/ml (Table 3).

The MIC and MFC values for the amphotericin B-susceptible (CL524) isolate were both 1 μg/ml with RPMI media; with AM3 the MIC and MFC were 0.015 and 0.06 μg/ml, respectively, and the Etest MIC was 0.38 μg/ml (Tables 2 and 3). Amphotericin B-resistant control isolate MY1012 had MIC and MFC values with RPMI media of 2 and 4 μg/ml, respectively; AM3 produced MIC and MFC values of >8 μg/ml, and the Etest MIC was >2 μg/ml (Tables 2 and 3). Amphotericin B-resistant control isolate CL2887 had MIC and MFC values with RPMI medium of 2 μg/ml; with AM3 media the MIC and MFC were both 1 μg/ml, and the Etest MIC was >2 μg/ml (Tables 2 and 3).

Identification of candidate interpretive breakpoints. Analysis of geometric means and median values of the results from each of the five susceptibility test methods showed no significant differences between the median values for isolates associated with treatment failures and successes (Table 4). The ROC curves produced for values obtained by the Etest MIC, AM3 MIC, AM3 MFC, RPMI MIC, and RPMI MFC methods differed substantially from an ideal test with high sensitivity and specificity (Fig. 1).

Because candidate interpretive breakpoints could not be identified with either of the previous two methods, we used published data to select breakpoints for resistance as follows: for broth microdilution MIC tests performed with RPMI 1640 medium, ≥2 μg/ml (4); and for broth microdilution MIC tests performed with AM3, ≥0.5 μg/ml (7, 9, 13). For Etest, a breakpoint of ≥0.38 μg/ml was chosen (4, 12). These breakpoints adequately differentiated between the susceptible and resistant control isolates in each respective susceptibility test method with the exception of the Etest. In this case, using the proposed breakpoint for resistance of ≥0.38 μg/ml, the susceptible control isolate, CL524, was misclassified as resistant. Using these breakpoints, none of the 107 bloodstream isolates from case-patients were classified as resistant according to MICs

TABLE 3. Distribution of amphotericin B MICs as determined by Etest

MIC dilution (μg/ml)	No. of *Candida* isolates					
	C. albicans	*C. glabrata*	*C. parapsilosis*	*C. tropicalis*	Others[a]	Total[b]
0.094	3	0	0	0	2	5
0.125	15	0	0	0	1	16
0.19	23	2	1	0	1	27
0.25	8	3	3	4	0	18
0.38	1	7	6	7	0	21, S
0.5	1	8	2	1	0	12
0.75	0	3	1	0	1	5
1	0	0	0	0	1	1
1.5	0	0	0	1	0	1
2	0	1	0	0	0	1
>2	0	0	0	0	0	0, R1, R2
Total	51	24	13	13	6	107

[a] *C. krusei* (*n* = 2), *C. dubliniensis* (*n* = 2), and *C. lusitaniae* (*n* = 2).
[b] R1, amphotericin B-resistant control isolate MY1012; R2, amphotericin B-resistant control isolate CL2887; S, amphotericin B-susceptible control isolate CL524.

TABLE 4. Geometric mean and median values of amphotericin B MICs and MFCs for 107 patients treated with amphotericin B, grouped according to treatment success or failure

Test method	Geometric mean (μg/ml)		Median (μg/ml)		*P*
	Failure	Success	Failure	Success	
RPMI 1640 MIC	0.33	0.32	0.5	0.5	0.9
RPMI 1640 MFC	0.83	1.05	1	1	0.2
AM3 MIC	0.015	0.016	0.06	0.06	0.7
AM3 MFC	0.085	0.10	0.125	0.125	0.4
Etest MIC	0.15	0.14	0.25	0.25	0.5

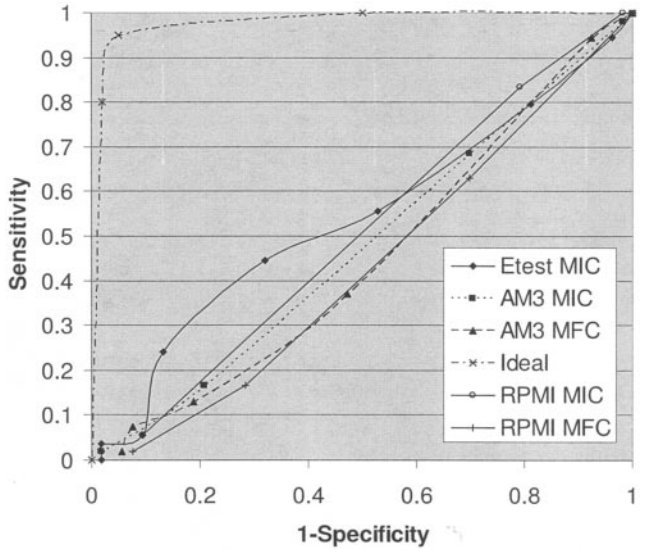

FIG. 1. ROC curve for amphotericin B susceptibility test results, as related to treatment failure, for 48-h Etest MIC and broth microdilution MIC and MFC using AM3 and RPMI medium.

determined in either RPMI 1640 or AM3 media; 24 (22%) were determined to be resistant by RPMI MFC, 17 (16%) were determined to be resistant by AM3 MFC, and 51 (48%) were determined to be resistant by Etest.

Correlation of in vitro susceptibility testing results with treatment failure. In vitro resistance as determined by RPMI MFC, AM3 MFC, or Etest did not significantly correlate with treatment failure on univariate analysis (Table 5). Being an infant was protective against treatment failure (relative risk [RR] 0.2, 95% confidence interval [CI] = 0.07 to 0.5), whereas case-patients with a central venous catheter (RR undefined), neutropenia (RR 2.0, CI = 1.4 to 2.7), or autoimmune disease (RR 1.6, CI = 1.1 to 2.4) at the time of candidemia were significantly associated with treatment failure.

Upon multivariable analysis controlling for age, APACHE II score, the presence of a subsequent bacteremia, *Candida albicans* versus non-*albicans* isolate, and catheter removal, in vitro resistance by any of the test methods was not significantly associated with treatment failure. We subsequently expanded the cohort to include those who had been treated for >3 days with amphotericin B monotherapy, but we were still not able to correlate in vitro resistance with treatment failure using these breakpoints (data not shown). We were also unable to find a correlation when the cohort was stratified to include only those treated with conventional amphotericin B (data not shown).

Epidemiologic characteristics of candidate interpretive breakpoints. The sensitivity, specificity, PPV, and NPV were calculated for each susceptibility test method to determine their ability to predict treatment failure using candidate breakpoints. As shown in Table 6, the specificity ranged from 68 to 94%, but the sensitivity was only 13 to 44%; NPV and PPV ranged from 38 to 59%.

DISCUSSION

Although it is clear that treatment failure in patients with deep-seated candidiasis is often due to factors other than elevated MIC, such as sequestered sites or catheter involvement, published reports demonstrate the potential for variation in amphotericin B MICs among *Candida* isolates and indicate that microbial resistance can develop during treatment in some patients (5, 11, 16). However, with few exceptions, attempts to correlate in vitro susceptibility to amphotericin B with outcomes in patients with candidiasis or animal models of infection have been unsuccessful (1, 9, 15). Rex et al. (15) found no correlation between amphotericin B MICs and the outcome of 66 patients with *Candida* bloodstream infections. Indeed, more cases of failure were seen among the patients infected with isolates for which MICs were low than among those infected with isolates for which MICs were high. In contrast, Nguyen et al. (9) demonstrated a good correlation between amphotericin B MICs and MFCs and the microbiologic response of 105

TABLE 5. Univariate predictors of treatment failures among 107 patients with *Candida* bloodstream infections and correlations of in vitro amphotericin B susceptibility interpretations with clinical outcome

Characteristic	No. (%) of:		RR (95% CI)	*P*
	Failures	Successes		
Infant	4 (12)	29 (88)	0.2 (0.07–0.5)	<0.01
Neutropenia	13 (87)	2 (13)	2.0 (1.4–2.7)	<0.01
Central venous catheter	52 (56)	41 (44)	Undefined	0.02
Autoimmune disease	9 (75)	3 (25)	1.6 (1.1–2.4)	0.05
Malignancy	14 (70)	6 (30)	1.5 (1.0–2.2)	0.05
All central venous catheters removed	46 (57)	35 (43)	1.1 (0.6–2.1)	0.4
APACHE II[a] score of >18	23 (56)	18 (44)	1.2 (0.8–1.7)	0.4
C. albicans isolate	22 (49)	23 (51)	0.9 (0.6–1.3)	0.6
Resistant by BM RPMI MIC[b]	0 (0)	0 (0)		
Resistant by BM RPMI MFC[b]	9 (38)	15 (63)	0.7 (0.4–1.2)	0.2
Resistant by BM AM3 MIC[c]	0 (0)	0 (0)		
Resistant by BM AM3 MFC[c]	7 (41)	10 (58)	0.8 (0.4–1.4)	0.4
Resistant by Etest MIC[d]	24 (58)	17 (41)	1.3 (0.9–1.8)	0.2

[a] APACHE, acute physiology and chronic health evaluation.
[b] ≥2 μg/ml.
[c] ≥0.5 μg/ml.
[d] ≥0.38 μg/ml.

TABLE 6. Analysis of candidate interpretive breakpoints for five amphotericin B susceptibility test methods used for analysis of in vitro-in vivo correlation

Test	Breakpoint (μg/ml)	%			
		Sensitivity	Specificity	PPV	NPV
Etest MIC	≥0.38	44	68	59	55
RPMI MIC	≥2	0	100		50
RPMI MFC	≥2	17	72	38	46
AM3 MIC	≥0.5	0	100		58
AM3 MFC	≥0.5	13	81	41	48

patients with *Candida* bloodstream infections. Although these isolates demonstrated a restricted range of MICs (0.06 to 2.0 μg/ml), the MFC range was much broader (0.125 to >16 μg/ml). Isolates from patients that experienced microbiologic failure showed significantly higher amphotericin B MFCs than those from patients that responded to treatment (9).

In this investigation, we used isolates and clinical data derived from a population-based surveillance program in an attempt to correlate in vitro susceptibility test results for amphotericin B with in vivo outcomes among patients with *Candida* bloodstream infections treated with this agent. As has been reported elsewhere (9, 15), we found that the CLSI broth microdilution reference method using RPMI 1640 medium produced a restricted range of MIC results. This narrow range of values suggests that amphotericin B susceptibility testing with this medium has limited clinical usefulness, especially since this method generally has an error of plus or minus one dilution (13). Broth microdilution MICs and MFCs obtained from tests using AM3 medium, as well as Etest MICs, spanned a broader range of values, a finding consistent with results from other studies (2, 4, 9, 13).

Using proposed breakpoints, we were unable to correlate in vitro susceptibility to amphotericin B with outcome using our clinical isolates. Patients with resistant isolates did not experience clinical treatment failure more frequently than those with susceptible isolates, even after multivariable modeling controlling for potential confounders such as age, severity of illness, catheter removal, the presence of a subsequent bacteremia, and the type of *Candida* species. Furthermore, the predictive ability of the different test methods, as determined by PPV and NPV, was not ideal for susceptibility testing. An example of this is the tests' failure to adhere to the "90-60 rule" (14). This rule suggests that infections due to susceptible isolates should respond to appropriate therapy ca. 90% of the time (i.e., NPV of 90%), whereas those due to resistant isolates should respond ca. 60% of the time (i.e., PPV of 40%). In the present study, none of the testing methods showed this level of predictive ability; MFC testing with AM3 and RPMI medium displayed adequate PPVs, but the NPVs were consistently near 50%.

One interpretation of why these methods did not correlate with treatment failure is that in vivo resistance may have been nonexistent. Indeed, using proposed breakpoints for MIC testing with RPMI and AM3 media, none of the isolates were resistant even though the amphotericin B-resistant control isolates had adequately high MICs. However, using MFC test results, which also differentiated between susceptible and resistant control isolates, in vitro resistance was frequent enough that a difference should have been detected.

Etest MIC results for isolates used in the present study were generally high. The susceptible control isolate had an MIC of 0.38 μg/ml, and 48% of study isolates had MICs of this concentration or higher. Although we were not able to find any suitable candidate breakpoints for defining resistance based on Etest MICs, a breakpoint of 0.38 μg/ml is likely to be too low.

Our definition of therapeutic failure may have not have been sensitive enough to detect a difference between sensitive and resistant isolates. Although mortality and fever are clinically useful indicators of treatment success, they are often strongly dependent on the hosts' underlying illness. Therefore, our definition of treatment failure may have been subject to overwhelming confounding, despite adjusting for severity of illness. The few studies that have successfully demonstrated in vitro-in vivo amphotericin B correlations for *Candida* bloodstream infections have used microbiologic outcomes (4, 9, 10), such as failure to clear the organism from the blood, to define therapeutic failure. However, in those studies, blood cultures were not performed systematically but were obtained at the discretion of the clinical care team. Therefore, both patients who failed and those who did not fail treatment were subject to information bias due to the inability to adequately categorize patients as treatment successes or failures. Microbiologic criteria for determining outcome were considered for our study, but thought not to be appropriate, since our retrospective design would be subject to the same limitations. A prospective study that collects serial blood cultures on all candidemic patients to systematically determine these outcomes would be very useful. Such a study could also detect the changes in MICs or MFCs in isolates exposed to amphotericin B in vivo.

In choosing our cohort of patients, we decided that >7 days of amphotericin B therapy was adequate to identify treatment failures. Some other studies that have been successful in showing an in vitro-in vivo correlation have included patients with >3 days of therapy (4, 9). However, expanding our cohort to include patients treated for 4 to 7 days with amphotericin B monotherapy still was not successful in showing any correlation. We also considered limiting the patient population to eliminate potential confounding factors, for example, by including only those who had all central venous catheters removed as part of treatment for their *Candida* bloodstream infection. However, since the removal of catheters was not significantly associated with failure on univariate analysis, controlling for catheter retention in the multivariable model was felt to be preferable to maintain the size of the study population.

One possible limitation of the present study may be that the cohort population was too small to detect a difference. However, since there was not even a trend toward statistical significance with the overall group, increasing the study population would likely not have resulted in different results.

Interpretive breakpoints for amphotericin B susceptibility testing have remained controversial due to conflicting results from correlation studies and, therefore, routine amphotericin B testing for *Candida* species isolated from the bloodstream is probably not indicated. Future prospective studies using broth microdilution MIC and MFC testing with AM3 medium or Etest with both clinical and microbiologic outcome measures may help with establishing guidelines for detecting amphotericin B resistance among *Candida* species.

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